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## Hall C, New Orleans Convention Center
## Coronary Artery Disease: Cellular Aspects

### PULSATILE STRETCH *IN VITRO* STIMULATES ENDOTHELIAL CELL PHOSPHATIDYL-INOSITOL TURNOVER

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We have previously reported that bovine aortic endothelial cells (EC) in culture respond to cyclic deformation with an increase in cell proliferation and prostacyclin synthetic activity. Since the phosphatidyl-inositol (PI) pathway has been implicated in the regulation of cellular growth and prostaglandin synthesis, the aim of the present study was to determine the effect of mechanical stretching of EC on PI turnover. EC ($2 \times 10^5$/ 25 mm) were grown on flexible-bottomed plates, labelled with $^3$H-myoinositol for 48 hours and then subjected to either 1, 10, or 100 cycles of 24% elongation-relaxation at 60 cycles/min. Control EC were subjected to similar incubation without stretch. Reactions were terminated with 15% trichloroacetic acid, and the inositol phosphates separated by anion exchange chromatography. The results are expressed as dpm/$10^5$ cells ± SD, *p<0.05 compared to control (0 cycle).

| Cycles | IP1 | IP2 | IP3 | IP4 |
|---|---|---|---|---|
| 0 | 584± 63 | 291± 65 | 205± 38 | 136± 67 |
| 1 | 679± 72 | 546±105 | 594± 69* | 180± 24 |
| 10 | 1124±139* | 686± 25* | 396± 59 | 204± 9 |
| 100 | 436±101 | 454± 29 | 345± 62 | 206± 21 |

Peak levels of $^3$H-inositol triphosphate (IP3) occurred rapidly and preceded that of the biphosphate (IP2) and monophosphate (IP1). Inositol tetraphosphate (IP4) was not significantly increased even at 100 cycles. Thus, cyclic deformation of EC stimulates PI turnover. PI metabolites are available to mediate some of the effects of cyclic stretch on the vascular endothelium.

### RELAXATION OF HUMAN INTERNAL MAMMARY ARTERY RINGS BY NEUTROPHILS: EVIDENCE FOR GENERATION OF AN EDRF-LIKE COMPOUND

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Recent studies have demonstrated that human polymorphonuclear leukocytes (neutrophils) generate nitric oxide when incubated at 37°C (BBRC 1989; 160:813-819). To determine the effect of neutrophils on human vascular smooth muscle tone, internal mammary arteries (IMA) were obtained from 15 patients undergoing coronary bypass surgery. The IMA was cut into 3mm rings and contracted with $TxA_2$ analog U46619 (mean tension 1.20±0.20g/mg tissue). Following stable contraction, isolated human neutrophils were suspended in a tissue bath maintained at 37°C. Neutrophils ($10^7$cells/ml) caused a gradual but consistent relaxation of IMA rings (mean decrease in tension 29±3%). Pretreatment of neutrophils with the superoxide anion scavenger superoxide dismutase (SOD, 200µg/ml), which inhibits the degradation of nitric oxide by superoxide radicals, potentiated this relaxation (66±4% vs 29±3%, P<0.01). Presence of the cyclic GMP inhibitor oxyhemoglobin (10µM), which inhibits nitric oxide-mediated vasorelaxation, resulted in conversion of neutrophil-induced IMA relaxation to contraction (-29±3% to +57±5%, P<0.01). Indomethacin (10µM) did not affect neutrophil-induced vasorelaxation, indicating that prostaglandins are not responsible for smooth muscle relaxation. These studies demonstrate that human neutrophils relax IMA. The vasorelaxant activity of neutrophils is potentiated by SOD and inhibited by oxyhemoglobin, suggesting that neutrophils produce a compound with physiochemical properties similar to those of the endothelium-derived vasorelaxant factor. Thus, circulating neutrophils may play an important role in the regulation of vascular tone.

### TISSUE-TYPE PLASMINOGEN ACTIVATOR RELAXES HUMAN CORONARY AND MAMMARY ARTERIES: A NEW ENDOTHELIUM-DERIVED RELAXING FACTOR?

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Tissue-type plasminogen activator (t-PA) is produced by endothelial cells and a potent fibrinolytic substance. Its vascular actions remain undefined. We studied the vascular effects of t-PA in the human left anterior decending coronary artery (LAD) and internal mammary artery (IMA) obtained intraoperatively. The artery rings were suspended in organ chambers filled with physiological salt solution for isometric tension recording. IMA and LAD were contracted with norepinephrine ($3 \times 10^{-7}$M) and prostaglandine $F_{2\alpha}$ ($3 \times 10^{-7}$M) respectively. Relaxations induced by t-PA ($10^{-10}$ to $10^{-8}$M) were then recorded. In IMA with endothelium, the relaxations induced by $10^{-9}$M and $10^{-8}$M t-PA were 26.8±15.3% and 73.9±9.6% respectively (n=6). In LAD, relaxations induced by $10^{-9}$M and $10^{-8}$M t-PA were 6.8±3.4% and 56.9±2.2% respectively (n=3). Removal of the endothelium did not significantly affect the responses in IMA (relaxations induced by $10^{-9}$M: 13.2±4.3%; $10^{-8}$M: 53.5±5.6%; n.s; n=6). Methylene blue ($10^{-5}$M) to inhibit guanylate cyclase in IMA also did not prevent the relaxations induced by t-PA ($10^{-9}$M: 19.8±9.3%; $10^{-8}$M: 54.7±14.8%; n.s; n=4). In conclusion, t-PA is a potent vasodilator of the human mammary and coronary artery. The relaxations are endothelium-independent and - in contrast to those evoked by endothelium-derived nitric oxide - not mediated by an increase of intracellular cGMP. Thus, t-PA may also act as an endothelium-derived relaxing factor preventing vasospasm.

### STIMULATION OF PLATELET PROSTAGLANDIN $E_1$ BINDING BY INSULIN IN MAN.

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Hyperactive platelets (PLT) in acute ischemic heart disease (AIHD) show impaired response to prostaglandin $E_1$ (PGE1), due to decreased PGE1 receptor numbers. To enhance PGE1 binding, normal platelet-rich plasma (n=40) was incubated with insulin (INS, 100 µU/ml, 2.5 h, 23°C), and at equilibrium, $^3$H-PGE1 binding (15 min, 23°C) was measured. PLT, exposed to INS, showed increased specific PGE1 binding from 275±41 fmol/$10^8$ PLT to 605±65 fmol/$10^8$ PLT (p<.001), decreasing minimum inhibitory concentration (MIC) of PGE1 for PLT aggregation (36±15 nM to 15±10 nM). Intravenous infusion of INS (50 mU/kg/h)-glucose-potassium for 2.5 h also increased the PGE1 binding from 260±30 fmol/$10^8$ cells to 760±110 fmol/$10^8$ cells (p<.001) in normal volunteers (n=15, between 21-35 years; mean plasma INS, approx. 130 µU/ml). The MIC of PGE1, which was 30±12 nM before the infusion decreased to 12±11 nM after the infusion. INS infusion also decreased the ability of ADP to aggregate these PLT (from 3±1.5 µM before the infusion to 10±2.5 µM after the infusion). These results suggest that INS enhances PGE1 binding to PLT and might reduce the hyperactivity of these cells in AIHD.